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Mycoses in Fish - A Survey of the Literature

By J. Dahle

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Fungi play an important role in diseases of fish. The pathogenic species are mainly ones which are specialized for life in water. These aquatic fungi may be saprophytic or parasitic, and their significance as pathogens is not entirely understood. The familiar diseases such as saprolegniasis, whirling disease and gill rot are discussed below, with a description of the pathogen and a survey of the available literature. The last section deals with the less frequently described diseases, including those caused by Exophiala salmonis, Scoleco-basidium humicola, Phoma herbarum and yeast fungi.

Saprolegniasis

The family Saprolegniaceae belongs to the class Oomycetes (Ainsworth, 1971 and 1973; Nolard-Tintigner, 1973). Most members of this family are saprophytic aquatic fungi which may occasionally also be found in moist earth (Esser, 1976). Reproduction can be either asexual or sexual. The first stage is development of sporangia at the hyphal apex which form motile, biflagellate zoospores and release them into the environment. These

zoospores emerge from their cysts, new biflagellate spores develop and germinate to a colony. This process can occur once or twice, depending on the species. The organs of sexual reproduction form at the tips of the hyphae or on short lateral branches. Swellings are formed by the accumulation of cytoplasm and vegetative hyphae are cut off from these by the formation of septa (Nolard-Tintigner, 1973). Vacuoles form inside the swelling which eventually subdivide the cytoplasm into several equal parts, giving it a stellate appearance. The organ thus formed is the female oogonium, in whose cell nuclei meiosis occurs. Every compartment of the cytoplasm surrounds itself with a membrane, and the haploid oospheres are formed. At the same time the antheridium (organ in which the male gametes are formed) is cut off by a septum from the same or a neighboring hypha.

Meiosis also occurs in the antheridium. The antheridium then makes contact with the oogonium and sends out a fertilization tube for every oosphere. Every tube transports a male nucleus which fertilizes an oosphere. The product of this union is called an oospore and surrounds itself with a thick cellulose membrane. Under favorable conditions these oospores can p.351 live for months, even without germinating (Nolard-Tintigner, 1973).

The morphology of the sex organs is used to identify the genera and species of saprolegniae. The two most important genera of this family are Saprolegnia and Achlya. The saprolegniae may be isolated from water samples or infected fish by

the bait method. A useful procedure is the culture of the fungi on hemp seeds (Cannibus sativa) (Johnson, 1956, and Seymour, 1970). Nolard-Tintigner (1973) uses split, cooked hemp seeds to isolate fungi in pond water samples (incubation with 20 ml water in a petri dish). Mycelium from lesions in infected fish can also be inoculated onto similarly prepared hemp seeds in 20 ml of distilled water. After 4 to 5 days, the seeds are surrounded by mycelium. The author also tried to grow the saprolegniae on Arbutin agar, brain-heart infusion agar (Difco), corn meal agar (Difco), carbon base (Difco), Czapek Dox agar, glucose peptone agar, maltose peptone agar, nitrogen base (Difco) and potato-carrot agar. According to her findings, however, sexual reproduction is infrequent in culture.

Fungi of the family Saprolegniaceae have often been associated with diseases in fish, but the authors do not agree on the significance of infection. Hofer (1904) points out the prevalence of fungal infections in fish, but takes the view that they are secondary infections. Drew (1909) observed lesions in salmon and detected gram-negative bacteria which he differentiated as Bacillus salmonis pestis. He also isolated Saprolegnia ferax. Stevenson (1970) believes that furunculosis (ulcerative dermal necrosis = UDN) starts with a viral infection. Roberts, Shearer and Munro (1972) and Roberts (1972) indicate the significance of the saprolegniae as a cause of secondary infection.

Nolard-Tintigner (1973) has isolated 43 strains of saprolegniae from infected fish over the course of several years. Exact differentiation was possible for 14 strains, including one strain of Saprolegnia delica, 6 strains of S. diclina, 6 of S. ferax and one of S. parasitica. White (1973) examined a total of 685 spawning sea trout (Salmo trutta) for saprolegniae, of which 453 (66%) were positive. Many of the infected fish were very debilitated and were found dead a few days after release. Most of the infected fish had scars or lesions. Neish (1977) describes lesions of fish skin with blisters and ulcers, some associated with wounds which were probably mechanical in origin. The symptoms were found in the head region of sockeye salmon (Onchorynchus nerka), and changes were also seen on the various fins as well. Histopathological examination showed first a destruction of the epidermis, with the fungus growth sometimes going through several layers and into the musculature. The author isolated Saprolegnia spec. by culture. Pickering and Willoughby (1977) observed white skin lesions in perch (Perca fluviatilis L.) and carried out histological studies with samples of skin and underlying muscle tissue. The lesions were divided into first, second, third and fourth degree. First degree lesions consisted of white areas 3-5 mm in size which occasionally showed small hemorrhages. Histological examination revealed loss of the epidermis.

Second degree lesions were larger (5-8 mm ø) and appeared as white depressions in the skin. Histological examination revealed loss of the epidermis. A few of the mostly

superficial melanophores showed signs of total breakdown with scattering or loss of the pigment granules. The melanophores (pigment cells) between the stratum compactum and the subcutis were contracted. The superficial muscle layers and the subcutis were infiltrated with erythrocytes and leucocytes.

Third degree lesions appeared as open wounds on the flank of the fish, with frequent signs of a fungus infection in the centre of the lesion. Those melanophores which were still intact were contracted. There were signs of necrosis in the stratum spongiosum. The tips of the scales were eroded. The superficial muscle layers and the subcutis had been massively infiltrated by blood cells.

In fourth degree lesions, the profuse growth of the fungus mycelium, which extended from the dermis into the subcutis, was the dominant feature. Many scales in the area of the lesions had been lost. Most of the superficial melanophores had disintegrated. In some cases the muscle fasciculi showed distinct signs of necrosis. No fungi could be cultured from 10 samples from first and second degree lesions. Leptomites lacteus was detected in two samples. Only in third and fourth degree lesions was it possible to detect fungi of the genera Achlya, Aphanomyces and to a lesser extent Saprolegnia. Some cases involved mixed infections of these fungi. Nolard-Tintigner (1973) also observed infections in perch. In every case, the dorsal region was affected as well as the caudal fin, in which the rays were often destroyed. Saprolegnia ferax was detected

by culture. McKenzie and Hall (1976) describe skin ulcers in thick-lipped mullet (Mugil cephalus). All fish displayed varying numbers of round or oval, dark red, flat ulcers. histological examination showed no remaining epithelium. The surface was necrotic and contained hemorrhages. A disease of fungal origin was indicated by the detection of hyphae. The authors assume that the causative agent was a member of the saprolegnia family.

Several authors have undertaken infection experiments in fish to establish the significance of saprolegniae as infectious agents. Tiffney and Wolf (1937) investigated the infectivity of a strain of the genus Achlya in 25 injured and 25 uninjured fish under bacteria-free conditions. Nine of the 25 injured fish became infected, while none of the uninjured showed symptoms. Vishniac and Nigrelli (1957) were able to cause infections with various species of saprolegniae in injured southern platyfish (Xiphophorus maculatus). Nolard-Tintigner (1973) was able to infect guppies and swordtails with saprolegniae. Shrivastava and Shrivastava (1977) did infection experiments on giant gourami (Colisa fasciata) and showed that Achlya diffusa, A. flagellata, A. orion, A. dubia, A. prolifera, A. carolinia, Isoachlya anisospora var. indica, Saprolegnia ferax, S. declina, Dictyuchus anomalus, D. strile, Aphanomyces laevis and a species of Aphanomyces which does not form sex organs are infective under laboratory conditions. Scott and Warren (1964) experimentally infected tropical fish (swordtail, guppy,

black molly and kissing gourami) with the genera Achlya, Saprolegnia, and Pythium. Achlya americana showed no particular p.352
specificity for the various species of fish. Growth of mycelium was visible after 7-10 h on artificially injured fish, and all fish died within 24 h. Of 8 uninjured fish, 2 showed symptoms of a mycosis and died. The results were similar for the genus Saprolegnia, except that the fish died only after 29 h. Of the uninjured fish, two died of mycosis, and a third died of unknown causes. The genus Pythium was found to be less pathogenic. Infection occurred in only 13 of 18 injured fish.

Whirling disease

The causative agent of whirling disease is Ichthyophonus hoferi (Phycomycetes, Chrytridiomycetes)(Schäperclaus, 1979), a fungus which grows in the organs of fish, characteristically forming a cyst in the host animal. The fungus spreads in the tissues via tubules which form connections to the developing daughter cells. In this way, the contents of the mother cell can be distributed to the daughter cells. The microscopic appearance of I. hoferi is spherical or egg-shaped with finely granulated protoplasm. The cysts, in which the fungus is usually found, can attain a size of 0.5-2.0 mm. According to Nehresheimer and Clodi (1914) the size varies from 110-210 µm. Schäperclaus (1979) has compiled the observations of several authors and defined the following stages of development:

- Breakdown of the orally ingested cysts in the digestive tract and formation of a small one-cell stage (ameboblast, small plasmodium)

- active penetration of the intestinal epithelium, entry into the circulatory or lymphatic system and transport to various organs
- formation of a cyst wall and enlargement by a series of nuclear divisions which take place when a certain size is attained, strengthening of the cyst wall and encapsulation with connective tissue.
- emergence of multinucleate plasmodia or sprouting of daughter cysts after a latent period of 8-14 days; the plasmodia can divide and form new cysts, thus overwhelming the affected organ.
- older parasites (cysts) are able to form resting spores or endospores.

According to Sindermann (1957) and Schlacher (1972), broth-gelatin agar, Sabouraud agar or serum substrates can be used to detect I. hoferi by culture.

Development of I. hoferi in the animal manifests itself pathomorphologically. After encapsulation of the cysts in the tissues, granulomas develop. The internal organs of diseased fish, especially the liver, may develop spherical nodules. Disturbances of motor coordination have been reported in trout and herring, thus giving rise to the name of the disease. The literature contains descriptions of the disease in various species of fish. Cox (1916) describes it in herring (Clupea harengus), Ellis (1928) in the winter flounder (Pseudopleuronectes americanus), Fish (1934) in the alewife (Alosa pseudoharengus), Rucker and Gustafson (1953) in the rainbow trout (Salmo gairdneri) and Powles et al. (1965) in the yellowtail

flounder (Limanda feruginea). Hendricks (1972) described for the first time the appearance of Ichthyophonus hoferi in salt-water fish in the north-west Atlantic, and detected the characteristic cysts in the kidneys of longhorn sculpin (Myoxocephalus octodecemspinosus) and cod (Gadus morhua). Pneumaticos (1977) reports on a severe case of ichthyophonus infection affecting young rainbow trout (Salmo gairdneri).

Gill rot

The causative agent of gill rot was first described by Plehn (1912). It is a fungus which grows in the branchial blood vessels. To the first species, Branchiomyces sanguinis, was later added Branchiomyces demigrans, first described by Wundsch (1929). Species differentiation is possible because only B. demigrans is able to penetrate the vessel walls and grow on the gill surfaces. Judging by the site of growth, B. sanguinis in particular requires much oxygen to grow. The fungus has so far not been detected in other organs than the gills. It is assumed that the infection starts from spores disseminated into the water from the necrotic gills of infected fish. Keiz (1959) suggests the following as possible predisposing conditions: elevated water temperature, high content of organic nutrients in pond water (e.g. from water fowl), worsening of conditions favoring water bloom, followed by mass die-off of organisms responsible for water bloom, high density of fish, which is especially the case if they are fed.

Grimaldi et al. (1973) give some suitable substrates for culture experiments. They cultured the fungus on fish agar and observed sporangium formation. Dankó et al. (1967) were able to culture it on maltose agar after Sabouraud. They describe thin brown, skin-like colonies which could easily be lifted from the substrate.

Gill rot was first observed only in carp and tench (Plehn, 1912). Outbreaks usually start suddenly and seem to depend on high external temperatures. Affected fish generally appear tired and listless and die very rapidly. The extremely short course of the disease and the irregular incidence has thus far considerably complicated investigation. In post mortem examination, the gills are noticeable for their speckled, dirty appearance. In more advanced cases, incipient lesions on the lamellar tips are seen. The cause of these signs becomes clear on histologic examination: the branchial blood vessels contain masses of hyphae which greatly reduce or completely prevent blood flow. Interruption of the oxygen supply leads to necrosis of gill tissue, usually distal parts. Spores are finally released into the surrounding water from these necrotic areas. Körting (1974) gives mortality rates of 10-50%, occasionally up to 100%. Following the earliest reports, gill rot has been observed in several species of fish. It appeared in pike and tench in another form (Wundsch, 1929). Since there was also fungal growth on the outside of the gills, the author called the causative agent Branchiomyces demigrans. Dankó et al. (1967) studied gill rot in siluroids and found superinfection

with saprolegniae. Grimaldi et al. (1973) observed the disease in bleak, rudd and coregonids in northern Italian lakes. The authors designate bleak and rudd as "indicator species" for gill rot. Grimaldi earlier (1971) describes cases in Alburnus alborella. Schäperclaus (1979) adds crucian carp, trout, whitefish, stickleback and Coregonus maraena / as possible hosts. His observations indicate that all age groups are susceptible, with young fish especially so. The greatest losses were seen in yearling and two-year-old carp.

Other mycoses

Following is a discussion of more rarely described mycoses. Carmichael (1967) isolated a previously unknown fungus in diseased Salmo clarkii which he named Exophiala salmonis spec. nov. The fish showed disturbances of equilibrium, and swimming movements were uncontrolled, degenerating into rapid turning motions in advanced stages. Shortly before death, swellings appeared on the head, the eyes protruded and ulcers appeared. In the first outbreak of the epidemic, 800,000 fish were infected, of which 480,000 (60%) survived. Post mortem examination revealed that the brain had been attacked first and the infection had spread from there to adjacent organs. Changes suggestive of mycetoma were observed, and infiltration with monocytes and lymphocytes was seen in the lesions. Necrotic areas were permeated with a network of hyphae. No lesions were discovered on eyes, gills, underjaw or any internal organ. p.353

Scolecobasidium humicola is a member of the Hyphomycetes.

Ross and Yasutake (1973) describe a systemic mycosis. It was outwardly manifest, if no skin changes were visible, by swelling of the abdomen. The body cavity was filled with a watery fluid. Deformities of the liver, spleen, kidneys and adjacent musculature also occurred. Gray lesions in the organs were similar to those seen in the kidney disease caused by corynebacteria. Histological studies revealed large lesions filled with hyphae and lymphoidal cells. The kidneys were affected especially often. Infection experiments were difficult, as the causative agent seems to be only weakly contagious. Infection of the rainbow trout (Salmo gairdneri) has also been described (Ajello et al., 1977). Blister-like areas appeared on the body surface, accompanied by edema and exophthalmus. In addition, bloody ulcers were spread over the entire body. The gills were sometimes anemic. Hemorrhages were observed in the internal organs such as liver and spleen, as were scattered dark-colored areas. There were hemorrhages throughout the body cavity involving the digestive apparatus and kidneys. Masses of mycelium could be seen on the organs. The histological picture was one of infiltration of lymphocytes and monocytes. In the diseased areas septate hyphae were found with an average diam. of 1.5 to 2.5 µm. The kidneys showed foci of inflamation with progressive replacement of the necrotic tissue by granulation tissue.

Ross et al. (1975) describe an infection with Phoma herbarum, a saprophyte which usually lives on plants. The fish had hemorrhages on the tail fin and petechial hemorrhages on

the abdomen and both sides. In the early stages of the disease, the fish swam on their side or vertically, with the tail directed downwards. In the final stage, they lay on the bottom on their side and only righted themselves when frightened. In the early stages, the air bladder contained mycelium which occasionally broke through and attacked the kidneys or other organs. When this happened, petechiae and necroses were found on the stomach wall, kidneys and gonads. The authors compared the histological findings with those in infection with Ecoleco-basidium humicola (Ross and Yasutake, 1973)

There are also reports of mycoses caused by yeast fungi. Cantoni et al. (1976) isolated several varieties of yeast in fresh water fish. Rhodotorula aurantiaca and R. graminis were the most frequent causes of infection. R. lactosa, Cryptococcus flavus, C. hungaricus and Saccharomyces spec. were isolated less frequently. Yeasts of the genera Torulopsis and Hansenula were also found. Pierotti (1971) was able to detect yeasts of the genus Crytptcoccus by culture. Natai and Ebusa (1975) report a candida infection in Oncorynchus rhodurus. They describe the major symptom as an extreme distension of the stomach. Candida sake was detected by culture. An extensive study of yeasts in marine fish was made by Bruce and Morris (1973). They isolated species of the genera Candida, Cryptococcus, Debaryomyces, Rhodotorula, Torulopsis and Trichosporon. The most frequently detected in culture was Debaryomyces hansenii.

Summary

This paper presents a synopsis of literature concerning fungal diseases in fresh water fishes and the marine ones. It is reported upon the etiology and the clinical and pathological aspects of Skin Fungus, Ichthyophoniasis Disease, Gill Rot and some less known diseases.

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